9:00 a.m.

870-3 **Metalloproteinase Inhibition Prevents Diastolic Stiffening, AMP-Breakdown, and Oxypurine Accumulation in Accelerated Heart Failure**

Nazareno Paolocci, Barbara Tavazzi, Roberto Biondi, Yehezkiel A. Gluzband, Mariangela Amorini, Carlo G. Tocchetti, Sonia Donzelli, Michael T. Crow, Giuseppe Lazzarino, David A. Kass, Johns Hopkins Medical Institutions, Baltimore, MD, University of Catania, Catania, Italy

Background: Recent studies indicated increased plasma oxypurine (hypoxanthine/xanthine/uric acid) levels as an independent risk factor for worsened heart failure, suggesting oxypurine potential role for diastolic dysfunction.
Methods: We used a canine model of enhanced diastolic failure (1 week AII infusion + subacute tachypacing; 250 bpm for 48 hrs; AII+P), displaying pronounced diastolic stiffening and marked metalloproteinase (MMP) activation. MMP inhibition (MMPI) prevents diastolic stiffening, without influencing collagen content/subtype or cross-linking. Here we tested whether AII+P (n=7) enhances AMP catabolism to increase cardiac nucleoside/oxypurine and diastolic stiffening, assessing the ability of MMPI to prevent these changes.
Results: AII+P raised diastolic chamber stiffness and end-diastolic pressure ~100% and markedly activated gelatinases MMP-9 and MMP-2 (abundance and *in situ* assays). With AII+P, ATP declined while AMP catabolites increased: nucleotides (inosine, adenosine) raised from 254±94 to 1700± 363nmol/g ($p<0.005$), and oxypurines from 100±20 to 666± 67 nmol/g ($p<0.001$). MMP inhibition (PD-166793, 5 mg/kg/day, n=9) prevented diastolic stiffening as well as MMP-9 and -2 activation, and countered the rise in both nucleotides (804± 35 nmol/g, $p<0.02$ vs AII-P) and oxypurines (397± 17, $p<0.001$ vs AII-P). MMPI directly inhibited *in vitro* AMP deaminase activity in a dose-dependent manner but did not affect other steps of the purine catabolism cascade. In AII+P hearts hallmarks of oxidative stress were evident: *malondialdehyde* (lipid peroxidation index) raised from 0.31±0.1 (controls) to 6.7±2.3 nmol/g ($p<0.01$) while antioxidant levels (ascorbate and reduced glutathione) significantly declined. However, MMPI did not mitigate AII+P-induced oxidative stress (MDA = 6.1±1.3).
Conclusions: Diastolic stiffening is associated with a rise in myocardial content of AMP-breakdown byproducts (i.e. nucleosides and oxypurines). MMP inhibition fully prevents diastolic stiffening by limiting this accumulation, direcly interfering with AMP catabolic activities (i.e.AMP deaminase), independently from the extent of cardiac oxidative stress.

9:15 a.m.

870-4 **Survival, Differentiation, and Contractility of Immature Cardiac Cells Implanted Into the Outer Wall of Aorta in Rats as a Step in the Development of an Auxiliary Circulatory Pump**

Wangde Dai, Sharon Hale, Robert A. Kloner, The Heart Institute, Good Samaritan Hospital, University of Southern California, Los Angeles, CA

Purpose: We proposed to build an auxiliary circulatory pump by implanting neonatal cardiac cells into the wall of aorta in rats. As a first step, we investigated the survival, differentiation and contractility of immature cardiac cells implanted into the wall of the abdominal aorta.
Methods: Cardiomyocytes from neonatal Fischer rats (both sexes) were injected into the outer wall of the abdominal aorta at a site 3 mm above the take-off of the renal arteries in female Fischer rats. Rats were divided into 2 groups: (1) medium only (n=22); (2) neonatal cardiomyocytes (n=22, 5×10^6 cells each). At 2 or 6 weeks, the grafted site on the aorta was exposed and fixed for histological and immunohistologic examination.
Results: At 2 weeks after transplantation, 7 out of 10 aortas in the cell group, but none of 10 in the medium group showed spontaneous rhythmic beating at the grafted site following excision of the heart. The intra-aortic pressure was comparable between the cell group and the medium group after the aorta was clamped at its origin (2.88 ± 0.45 vs 2.94 ± 0.63 mmHg, p= 0.94). Pacing in a cell grafted aorta increased aortic pressure 5 fold from baseline. PCR of the SRY gene to identify male cells was positive in 3 examined aortas in the cell group confirming presence of transplanted cells and none of 3 aortas in the medium group at 6 weeks. Hematoxylin and eosin staining showed viable grafts in the outer wall of the cell-treated aortas in 9 out of 10 aortas at 2 weeks and 9 out of 9 aortas at 6 weeks; and none of the aortas receiving medium at 2 (n=10) or 6 weeks (n=9). Neonatal cardiomyocytes in the graft formed compact, longitudinally oriented cardiac muscle bundles, and were differentiated with cross strations and a high degree of vascularization. Immunohistochemical staining for sacromeric actin was positive in 4 out of 10 aortas at 2 weeks and 9 out of 9 aortas at 6 weeks in the cell group; and none of the aortas in the medium group.
Conclusion: The results show that **g**rafted neonatal cardiomyocytes survive, differentiate, develop a blood supply and spontaneously contract within the outer wall of the aorta in rats. It is feasible to transplant immature cardiac cells into the aorta to fashion an external auxiliary circulatory pump.

9:30 a.m.

870-5 **Uncovering Human Cardiac Myocyte Progenitor Cells for Myocardial Regeneration**

Lincoln T. Shenje, Bashir M. Matata, Manuel Galiñanes, University of Leicester, Leicester, United Kingdom

Background: The failure to identify and culture human cardiac progenitor cells has reinforced the idea that the heart is a terminally differentiated organ, with little or no capacity for self renewal. Increasing evidence suggests baseline levels of myocyte loss in the human myocardium require sustainable myocyte renewal for cardiac homeostasis. We investigated whether cardiac progenitor cells can be cultured from the human myocardium.
Methods: Cells derived from cultured human right atrial tissue explants (RATEs) were depleted of fibroblast surface antigen (FSA) positive cells using immunomagnetic beads. The resultant FSA negative fraction was assessed for cells expressing markers of cardiac differentiation using flow cytometry and confocal microscopy as well as for stem cell markers and the cell cycle antigen Ki-67.
Results: Human atrial myocardial tissue explants produce a heterogeneous cell population amongst which are small highly proliferating cells, expressing markers of cardiac differentiation; Csx/Nkx-2.5, GATA-4, alpha-sarcomeric actinin, cardiac myosin heavy chain together with the cell cycle antigen Ki-67. In addition the cells also express c-Kit and MDR-1 denoting stem cell properties. At 9-12 weeks in culture they fuse to form rod like multinucleate cells similar to those previously described as myotubes.
Conclusions: Our study shows for the first time that there is a potential for self renewal of the human myocardium involving cardiac progenitor cells and that cardiac stem cells can be cultured. This has major implications in the study of stem cell biology and the development of cell therapy for myocardial regeneration.

9:45 a.m.

870-6 **The Role of Angiopoietin-1 in Physiologic and Pathologic Cardiac Remodeling and in Promoting Cardiac Myocyte Survival**

Susan Marie Dallabrida, Michelle Euloth, Maria Ann Rupnick, Children's Hospital, Boston, MA, Harvard Medical School Affiliate, Boston, MA

Background: Cardiac remodeling contributes to cardiovascular disease progression, emerging as a therapeutic target for heart failure.While remodeling initially compensates for injured myocardium, it is a progressive process that is ultimately maladaptive. Understanding processes resulting in adaptive growth are needed. In development, the heart grows and remodels via increases in myocytes (hypertrophy, hyperplasia) and capillaries (angiogenesis). We propose angiopoietin-1 (ang1), a vascular maturation factor, is a mediator of cardiovascular remodeling. Ang1 is required for cardiac development and increases in hypoxic myocardium. Ang1's receptor, tie2 is endothelial-specific, so its role is thought to be limited to vessels. A recent study shows that ang1 binds integrins, perhaps revealing new roles for ang1 in cardiac development and remodeling.
Methods: We measured ang1, ang2, and tie2 mRNA levels in developing/adult hearts (fetal, 1, 2, 4, 7, 14, 21, 56, 133, 238 d) and thyroid hormone (T3)-induced cardiac hypertrophy (1-4, 7, 14 d) using C57BL/6 mice, and in neonatal/adult cardiac myocytes and a cardiac myocyte cell line (C2C12) (real-time RT-PCR). We compared ang/tie in the left ventricle (LV), right ventricle (RV), and atria (AT) (*in vivo* studies). We examined if ang1 binds (cell adhesion) and promotes cardiac myocyte survival/proliferation using *in vitro* studies (C2C12 cells).
Results: Ventricular and atrial ang1 levels increase in the fetal to adult transition. Ang1 increases (LV=475, RV=75, AT=4 fold) are related to the extent that the chamber mass increases. Ang1 was undetectable in neonatal, but prominent in adult cardiac myocytes. T3-induced cardiac hypertrophy increases LV ang1 up to 20 fold. Cardiac myocytes bind ang1. Ang1 markedly promotes myocyte survival and proliferation in serum-free media (7 d versus controls < 1 d) with the number of surviving cells declining from 7-21 d.
Conclusion: Our studies support a role for ang1 in physiologic and pathologic cardiovascular remodeling. They identify a mechanism for enabling myocyte survival/proliferation in adverse conditions, and have broad implications for cardiac hypertrophy/heart failure therapy development.

ORAL CONTRIBUTIONS

872 Heart Failure: Resynchronization Therapy

Wednesday, March 10, 2004, 8:30 a.m.-10:00 a.m.
Morial Convention Center, Room 260

8:30 a.m.

872-1 **Reverse Remodeling With Cardiac Resynchronization Therapy Varies With Infarct Location: Analysis of Echocardiographic Data From the MIRACLE Trial**

Martin St. John Sutton, Ted Plappert, Thomas J. Mullen, Kathyrn Hilpisch, Edward Chinchoy, University of Pennsylvania Medical Center, Philadelphia, PA, Medtronic, Inc, Minneapolis, MN

Reverse remodeling has been reported in moderate to severe heart failure (HF) patients (pts) with ventricular dysynchrony after cardiac resynchronization therapy (CRT). This analysis assessed whether infarct location predicted the degree of reverse remodeling after 6 months of CRT. **METHODS:** The MIRACLE study enrolled NYHA Class III/IV HF pts with ventricular dysynchrony and LVEF$\leq$35%. All pts were implanted with an InSync atrial synchronous biventricular pacing system. AV delay was individually optimized and pts were randomized to no pacing (control) or to CRT. Doppler echocardiograms were analyzed by a core laboratory. Left ventricular end diastolic volume (LVEDV), LV end systolic volume (LVESV), LV ejection fraction (LVEF), and mitral regurgitation color flow jet area (MR) were calculated. Infarct location was determined using a 16 segment model. Anterior infarction (AMI) was defined as akinesis in at least 3 antero-apical segments and inferior infarction (IMI) defined as akinesis in at least 3 postero-inferior segments. Changes in LV remodeling were quantified and compared between the AMI and IMI groups after 6 months of CRT. **RESULTS:** Significant reductions in LVEDV and LVESV

occurred in the IMI group but not in the AMI group. Improvements in LVEF and MR were similar in both groups. **CONCLUSION:** Infarct location determines the extent of reverse LV remodeling in HF pts treated with CRT independent of baseline LV volume and function.

	AMI		IMI	
	Baseline Mean ± Std	**6mo. Median Paired Difference, (95% C.I.)**	**Baseline Mean ± Std**	**6mo. Median Paired Difference, (95% C.I.)**
LVEDV, cm^3	297.6 ±97.4 (n=48)	1.6 (-23.4, 20.7)	307.8±94.6 (n=28)	-43.2 (-69.0, 0.4)†*
LVESV, cm^3	229.6±89.5 (n=48)	-5.4 (-18.6, 3.7)	235.1±82.3 (n=28)	-24.1 (-66.6, -11.3)†*
LV EF, %	24.1±6.2 (n=48)	2.3 (0.8, 3.9)†	24.6±6.8 (n=28)	3.2 (0.2, 4.5)†
MR Jet Area, cm^2	9.35±4.88 (n=36)	-4.2 (-5.8, -2.6)†	8.20±5.32 (n=17)	-4.0 (-5.9, 2.3)†

* p<0.05 AMI vs IMI
† p<0.05 within group changes, baseline to 6-months

8:45 a.m.

872-2 **Upgrade From Standard Right Sided Pacing to Cardiac Resynchronization Therapy Shows Clinical Benefit as in De Novo Implantation**

Salpy V. Pamboukian, Imran Nisar, Sheetal Patel, Liping Gu, Mary McLeod, Stephanie Dunlap, Richard Trohman, Maria Rosa Costanzo, Alain Heroux, University of Alabama at Birmingham, Birmingham, AL, Rush Presbyterian St. Luke's Medical Center, Chicago, IL

Background: Cardiac resynchronization therapy (CRT) produces clinical benefit in heart failure (HF) patients (pts) with intraventricular conduction delay. Previous studies excluded pts who already had a pacing device. In this study, pts with denovo (DN) CRT were compared to those undergoing upgrade (UP) to CRT from standard pacing.
Methods: Data from the Rush Heart Failure Database was collected including demographics, cause of HF, NYHA class, echo data (left ventricular ejection fraction LVEF, LV end diastolic dimension EDD, mitral regurgitation MR, tricuspid regurgitation TR), ECG intervals, drugs, hospitalizations (hosp), length of stay (LOS), procedure complications and mortality. Statistics were performed using t test, Fisher's exact test, Wilcoxon test.
Results: Twenty eight pts had DN implant (19 male, 13 ischemic, LVEF 20%, LVEDD 72mm), 21 pts had UP (15 male, 13 ischemic, LVEF 24%, LVEDD 73mm). Age was 61±9 yrs in DN versus (vs) 67±10 in UP (p<0.01). PR interval was 188±34ms in DN vs 156±57ms in UP. QRS duration was 152±21ms in DN vs 181±35ms in UP (p<0.01). In the DN, LVEDD decreased by 4±2mm after CRT (p=0.04). In the UP, MR was less after CRT(p=0.02). Both groups had improved NYHA class immediately after CRT (p=0.02). In DN group this was still seen at 6 and 12 months (P<0.01). No difference was seen in the number of hosp in the groups 6 months before and after CRT. There was a trend to shorter LOS in the in the DN pts after CRT, -5± 2.5days(p=0.07). Dose of metoprolol succinate was 62±17mg at baseline in DN pts but increased by 53±19mg after CRT(p=0.01). Carvedilol dose was unchanged. No difference in beta blocker dose after CRT was seen in the UP pts. There were no differences in CRT related complications. There were 3 deaths in UP, 4 in DN group.
Conclusion: HF pts with standard pacing benefit from upgrade to CRT with improved NYHA class and decreased MR, without increased procedure related complications. CRT allows for uptitration of beta blockers in DN implants.

9:00 a.m.

872-3 **Reverse Remodeling After Cardiac Resynchronization Therapy and Analysis of Effect in Heart Failure Patients With or Without an Indication for Implantable Cardioverter Defibrillator**

Martin G. St. John Sutton, Ted Plappert, Kathryn E. Hilpisch, Edward Chinchoy, University of Pennsylvania Medical Center, Philadelphia, PA, Medtronic, Inc, Minneapolis, MN

Evidence of reverse remodeling with CRT in moderate to severe heart failure (HF) patients (pts) with ventricular dysynchrony has been reported in several trials. An analysis was done to compare the results of a CRT versus a CRT+ICD trial. **METHODS:** We compared the magnitude of remodeling of two separate CRT trials differentiated by pts with and without an indication for an ICD. The MIRACLE and MIRACLE ICD trials enrolled HF pts (NYHA III/IV, QRS>130ms, LVEDD >55mm and EF<35%) and implanted them with an InSync (atrial synchronous biventricular pacer) or an InSync ICD system, respectively. In all pts AV delay was echocardiographically optimized by maximizing left transmitral filling without truncating the A wave. Patients were randomized to no pacing (control) or to treatment (CRT or CRT+ICD). Peak VO_2 consumption (pVO_2), 6-minute hall walk distance (6'HW) and Quality of life (QoL) were compared. Doppler echocardiograms were recorded in pts at baseline and 6 months, and analyzed by a core laboratory. LV end diastolic volume (LVEDV), LV end systolic volume (LVESV), LV ejection fraction (LVEF), Deceleration time (DT), and mitral regurgitation color flow jet area (MR) were obtained. Only treatment patients with paired baseline and 6 mos data were used. **RESULTS:** See table. **CONCLUSIONS:** The magnitude of clinical improvements and reverse remodeling quantified using echocardiographic indices was similarly evident in both trials regardless of indication or placement of an ICD. *p<0.05 between baseline and 6 months.

mean ± std	MIRACLE (n=196)		MIRACLE ICD (n=165)	
	Baseline	6 mo	Baseline	6 mo
LVEDV (ml)	302±107	266±109*	318±95	299±101*
LVESV (ml)	233±98	195±99*	245±88	224±94*
LVEF (%)	24.2±6.8	29.2±9.0*	24.2±6.5	27.3±8.9*
MR (cm^2)	7.4±5.9	4.3±4.4*	7.7±6.0	6.4±5.4*
DT (ms)	199±84	236±108*	197±80	215±78*
pVO_2	14.3±3.5	15.4±4.1*	13.5±3.5	14.3±3.7*
6' HW (m)	310±83	335±125*	250±127	338±106*
QOL	59±20	39±24*	55±23	37±24*

9:15 a.m.

872-4 **Benefit of Biventricular Pacing in Subsets of Patients With Heart Failure**

Kenneth Ng, Navin Kedia, Randall Starling, Bruce Wilkoff, Pat Tchou, David Martin, Richard Grimm, Cleveland Clinic Foundation, Cleveland, OH

Background
Extending the current indications for cardiac resynchronization therapy (CRT) to patients with NYHA Class II symptoms, RV pacing or QRS ≤ 150ms has been proposed however no data has yet been reported to support this recommendation.
Methods
One-hundred and forty-four consecutive heart failure patients underwent CRT from January 1999 to January 2002. Clinical parameters of NYHA Class, QRS duration, LVEF, LV end-systolic (LVESD) and end-diastolic (LVEDD) dimensions were monitored at baseline and end of follow-up with a mean of 330 ± 220 days.
Results
At baseline, 20 patients, 88 patients and 36 patients were in NYHA Class II, Class III and Class IV respectively, 34 patients with right ventricle pacing, and 29 patients with a QRS duration ≤150ms. After CRT, the patients who were in NYHA Class II had significant improvement in LVEF and LV dimensions. Patients with RV pacing had significant improvement in NYHA Class, QRS duration, LVEF and LV dimensions. The patients with QRS duration ≤ 150ms had significant improvement in NYHA class and a significant increase in the QRS duration.(Table I)
Conclusion
Non-conventional candidates for CRT may derive significant structural and functional benefit with CRT and should be analyzed in future clinical trials.

Patient Subgroups	NYHA Class II		NYHA Class III/IV		RV Pacing		QRS duration ≤ 150ms	
Clinical Variables	Pre-CRT	Post-CRT	Pre-CRT	Post-CRT	Pre-CRT	Post-CRT	Pre-CRT	Post-CRT
NYHA Class	2	1.75	3.1*	2.5*	3.1*	2.5*	2.9*	2.5*
QRS Duration (ms)	171	159	174*	164*	195*	165*	137*	155*
LVEF (%)	17.9*	27.6*	18.9*	23*	18.1*	22.2*	22	22
LVEDD (cm)	6.6*	6.1*	6.8*	6.5*	6.4*	6.1*	6.6	6.5
LVESD (cm)	5.5*	4.8*	5.7*	5.3*	5.4*	5.1*	5.5	5.3

* denotes difference is significant, p<0.05

9:30 a.m.

872-5 **Incidence of Diastolic Dyssynchrony in Dilated Cardiomyopathy and Effects of Biventricular Pacing**

Christophe Jego, Iris Schuster, Christine Medail, Jean Lefevre, Frederic Franceschi, Jean-Marie Vailloud, Ange Ferracci, Jean-Claude DeHaro, Pierre Djiane, Gilbert H. Habib, La Timone Hospital, Marseille, France

Background. Resynchronization is an accepted treatment of dilated cardiomyopathy (DCM). Its benefit has been related to the correction of *systolic* (S) dyssynchrony (DYS) either interventricular (Inter) or intraventricular (Intra). However, little is known about *diastolic (D)* DYS and the effects of biV pacing on it
Objectives:
1 - to compare the respective occurrence of D and S DYS in pts with DCM and LBBB.
2 – to assess changes in both D and S delays under Biv pacing
Methods. 37 pts with DCM and wide QRS were studied by Tissue Doppler Imaging (TDI) before and immediately after biV pacing. S and D parameters were measured using delays between onset of QRS and onset of the TDI velocity curves. The septal and lateral walls of the left ventricle were used to assess Intra DYS. Similar measurements between lateral free walls of right and left ventricles were used to assess Inter DYS.